

Original Research Article

Phyto-chemical Screening and Antibacterial Activity of Five Indian Medicinal Plants against Human Pathogens

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ABSTRACT

Keywords

Indian medicinal plants; Phytochemical screening; antibacterial activity; Human pathogens

The medicinal plants represent an enormous reservoir of potential phytochemical compounds that could be useful as an alternative to synthetic microbicides and are being used to develop drugs. In the present study, five selected Indian medicinal plants such as *Andrograpis paniculata*, *Cassia alata*, *Cardiospermum halicacabum*, *nyctanthes arbortritis* and *solanum nigrum* were analyzed for phytochemical constituents and tested for antibacterial activity against human pathogens. In most of the samples all the phytochemicals i.e. reducing sugar, Terpenoids, Flavonoids, Saponins, alkaloids, cardiac glycosides, carbohydrates and phytosterols were present. However in some samples the anthraquinones and the tannins were absent. Methanol, ethyl acetate, chloroform and aqueous extracts of leaf samples of all plants were evaluated for *in vitro* antibacterial activity against *Escherichia coli*, *Klebsiella pneumoniae*, *Micrococcus luteus* and *Pseudomonas aeruginosa* by agar plate well diffusion method. All the plant extracts showed significant antibacterial activity against the tested organisms. However, methanol extracts showed maximum inhibitory effect from this *C. halicacabum* and *C. alata* were showed maximum bacterial inhibition than other plants.

Introduction

For centuries plant have been used throughout the world as drugs and remedies for various diseases since they have potential for producing new drugs of great benefit to mankind. There are many approaches to search for new biologically active principles in higher plants. One such approach is systematic screening, which

may result in the discovery of novel effective compounds. Despite the existence of potent antibiotics and antifungal agents, resistant or multi-resistant strains are continuously appearing, imposing the need for a permanent search and development of new drugs. This revival of interest in plant-

derived drugs are mainly due to the current wide spread belief that "Green medicine" is safe and more dependable than the costly synthetic drugs, many of which have adverse side effects. The purpose of this study was to screen some Indian medicinal plant extracts that could be useful for the development of new tools as antimicrobial agents for the control of infectious diseases.

Andrographis paniculata belongs to the family of Acanthaceae. It is an annual plant with characteristic white purple (or) spottle purple flowers. It grows height of 30-70 cm (kumaro and Masitah Hasan, 2007). Stem is dark green. Leaves are glabrous lanceolate, pinnate. Flowers are small, spreading auxiliary and terminal racemes. Capsules linear-oblong acute at bottom ends. *Andrographis paniculata* has been used for treatment of respiratory tract infection, tonsillitis, fever, diarrhea, inflammatory injury, herpes simplex and herpes zoster infection (Fujita *et al.*, 1989). Also used as an antidote for snakes, insects bite and as an antimalarial drug (Chakravati and Chakravati, 1989). It has antihelmintic, antipasmotic, antiperistaltic activity and also used for dyspepsia and colicdysentery (Dhiman Anju *et al.*, 2012)

Cassia alata Linn, belonging to family of caesalpiniaceae. Distributed from tropical America to India reaching a height of 10 to 12 feet with an equal spread leaves are simple pinnate, buds covered with orange bracts which fall off when the flower opens, fruit are black pod with two broad wings seeds are small, square and rattle shaped. It is used to treat a wide range of ailments from stomach problems fever, asthma to snake bite and venereal diseases (syphilis, gonorrhoea) the boiled leaves are

used for blood pressure in Africa. It is used to treat urinary tract and gastrointestinal tract disorders (Mahmood and Doughari, 2008). It is used for the treatment of constipation, inguinal hernia, intestinal parasitosis, syphilis and diabetes (Saheli Chatterjee *et al.*, 2012).

Cardiospermum halicacabum is a plant belongs to the family sapindaceae. It is a deciduous climber growing upto 3 meters. The ground stem carries alternate double triad leaves 3 to 6 cm long, the tiny radiate flowers. Stems are 5 to 10 cm in length. The fruits are tiny green balloon shaped; spherical capsule containing the characteristics seeds with their heart shaped white markings. It is used in the treatment of arthritis (Eugene Wilson *et al.*, 2007), nervous disease, stiffness of the limbs and snake bites. The leaves are applied as poultice in the treatment of rheumatism. The tea made from this is used in the treatment of itchy skin, salted leaves are used as poultice on swellings, and the leaf juice has been used as a treatment for earache. It is also used in the treatment of rheumatism, chronic bronchitis and stiffness of the limbs and snakebite (Huma Shareef *et al.*, 2012)

Nyctanthes arbortritis (night flowering jasmine) is a small tree growing up to 10 feet tall, with flaky grey bark. The leaves are opposite, simple, 6 to 12 cm long and 2 to 7 cm broad, with an entire margin. The flowers are fragrant, with a five to eight lobed white corolla with an orange-red centre. The fruits are flat brown heart shaped to round, capsule 20 mm in diameter with two sections each containing a single seed. Leaves, flowers and seeds are useful for various diseases such as chronic fever, bronchitis, asthma,

constipation, grayness of hair, baldness and skin diseases. The seeds of *Nyctanthes* used for treat the piles. Leaves used to treat the dry cough, ring worm. It has antipyretic effect (Saxena *et al.*, 1987) and anti inflammatory effect (Saxena *et al.*, 1984). The decoction of leaves is extensively used by Ayurvedic physicians for the treatment of arthritis, obstinate sciatica, malaria, intestinal worms and as a tonic, cholagogue and laxative (Meshram *et al.*, 2012).

Solanum nigrum belong to the family solanaceae. *Solanum nigrum* is an annual herbaceous plant of 10 to 60 cm height with a green smooth and semi climbing stem. The opposite leaves with whole limb, oval and diamond shaped. Both surfaces of leaves are hairy (or) hairless petiole 10-30 mm long with winged upper portion, the flowers have petals greenish to whitish, when aged and surround prominent bright yellow anthers. The berry is mostly 6 to 8 mm diameter, dull black (or) purple black. In India *solanum nigrum* mixed with other herbal medicine used for hepatoprotective activity (Sultana *et al.*, 1995). This plant also having diaphoretic, diuretic, expectorant and are useful in the disease of liver, heart and eyes and is also effective against piles, fever and dysentery (Rastogi and Mehretra, 1992). The berries have been used in the treatment of stomach ulcers in folk medicine (Ikram and Hussain, 1918). Considering the above features of these five Indian medicinal plants were investigated for their phytochemical constituents and antibacterial activity. The ethanolic extract of *Solanum nigrum* also exhibited the highest antifungal activity against *Microsporum gypsum*, *Trichophyton mentagrophytes*, *Epidermophyton floccosum* and *Aspergillus niger* (venkatesan *et al.*, 2009).

Materials and Methods

Collection of plant materials

The plants were collected from surrounding areas of Javvadu hills, Tiruvannamalai District, Tamilnadu state in India.

Preparation of plant extracts

Collected plants were cleaned, shade dried and ground as powder form. Then the samples were extracted by using different solvents (methanol, ethyl acetate, chloroform and water) in soxhlet apparatus and concentrated by using rotary evaporator.

Preliminary Phytochemical Screening

The methanolic extract of *Andrographis paniculata*, *Cassia alata*, *Cardiospermum halicacabum*, *Nyctanthes arbortritis* and *Solanum nigrum* was subjected to preliminary phytochemical screening of various constituents in the leaves.

Test for Alkaloids

Wagner's test

Wagner's reagent: Iodide, 1.2g and 2.0g of potassium iodide were dissolved in 5ml of sulphuric acid and the solution was diluted to 100 ml.

10 milliliters of the extract was acidified by adding 1.5% v/v of HCL and a few drops of Wagner's reagent. The yellow formation or brown precipitate confirmed the presence of alkaloid.

Mayer's test

Mayer's reagent: The mercuric chloride (1.36) was dissolved in 60ml of distilled

water and 5g of potassium iodide in 10 ml of water. The two solutions were mixed and diluted to 100 ml with distilled water. The extract (1.2 ml) was taken in a test tube to which 0.2 ml of dilute hydrochloric acid and 0.1 ml of Mayer's reagent was added. Formation of yellowish buff coloured precipitate confirmed the presence of alkaloids.

Test for Flavonoids

Shinoda's test

In a test tube containing 0.5 ml of extract 5-10 drops of dilute HCl and small piece of zinc chloride or magnesium were added and the solution was boiled for few minutes. In the presence of flavonoids, reddish pink colour was produced.

Alkaline Reagent Test

To 1.0 ml of the extract, a few drops of dilute sodium hydroxide were added. An intense yellow colour was produced in the plant extract, which become colorless on addition of a few drops of dilute acid, which indicates the presence of flavonoids.

Test for Carbohydrates

Molisch's test

A small quantity of the extract was dissolved separately in 4 ml of distilled water and filtered. The filtrate was subjected to Molisch's test to detect the presence of carbohydrates. Filtrate was treated with 2-3 drops of 1% alcoholic α -naphthol solution and 2 ml of conc. H_2SO_4 was added along the sides of the test tube. Appearance of brown ring at the junction of two liquids shows the presence of carbohydrates.

Test for Glycosides

The extract was hydrolyzed with HCl for few hours on a water bath and the hydrolysate was subjected to Legal's or Borntrager's test to detect the presence of glycosides.

Borntrager's test

Hydrolysate was treated with chloroform and then the chloroform layer was separated. To this equal quantity of dilute ammonia solution was added. Ammonia layer acquires pink color, shows the presence of glycosides.

Test for Saponins

The extract was diluted with 20 ml of distilled water and it was agitated in a graduated cylinder for 15 minutes. The formation of 1cm layer of foam shows the presence of saponins. The extract, 1 ml was treated with 1% lead acetate solution. Formation of white precipitate indicates the presence of saponins.

Test for Tannins

Ferric chloride test

To 1-2 ml of the extract and a few drops of 5% aqueous $FeCl_3$ solution was added. A violet colour formation indicates the presence of tannins.

Lead acetate test

In a test tube containing about 5 ml of the extract and a few drops of 1% lead acetate was added. A yellow precipitate was formed, indicates the presence of tannins.

Test for Phytosterol

The extract was refluxed with solution of alcoholic potassium hydroxide till complete saponification takes place. The mixture was diluted and extract with ether. The ether layer was evaporated and the residue was tested for the presence of phytosterol.

Salkowski test

The extract (10 mg) was dissolved in few drops of chloroform; 1 ml of Conc.H₂SO₄ was added carefully along the sides of the test tube. The red colour was formation indicate the presence of sterols.

Test for Triterpenoids

Libermann Burchard test

The extract, 10 mg was dissolved in 1 ml of chloroform; 1ml of acetic anhydride was added following the addition of 2 ml of Conc.H₂SO₄. Formation of reddish violet colour indicates the presence of triterpenoids.

Noller test

The extract, 5 mg was dissolved in 2 ml of 0.01% anhydrous stannic chloride in pure thionyl chloride. A purple colour formed then changed to deep red after few minutes and indicates the presence of triterpenoids.

Test for Proteins and Amino acids

Ninhydrin test

The extract (1 ml) was treated with few drops of Ninhydrin reagent. Appearance of purple colour shows the presence of amino acids.

Biuret test

Equal volumes of 5 % NaOH solution and 1% copper sulphate solution were added to 1.0 ml of the extract. Appearance of pink colour shows the presence of proteins.

Test for Anthraquinones

The extract solution (5ml) was hydrolyzed with diluted Conc.H₂SO₄ extracted with benzene. 1 ml of dilute ammonia was added to it. Rose pink colouration suggested the positive response for anthraquinones.

Bacterial culture collection

The pathogenic bacteria namely, *Escherichia coli* (MTCC 443), *Klebsiella pneumoniae* (MTCC109), *Pseudomonas aeruginosa* (MTCC 1688) and *Micrococcus luteus* (MTCC106) were obtained from Institute of Microbial Technology, sector 39-A, Chandigarh-160036, India.

Antibacterial activity assay

Muller-Hinton agar and Nutrient agar were poured on to sterile Petri plates. When the media solidified, 0.1ml of inoculum with 0.5 OD was poured over feeder layer and spread evenly with a sterile spreader. A well of 6mm diameter was made by using a sterile cork borer. Each well-received different concentration (50µl, 100µl and 150µl) of crude extract was tested in a concentration of 100 mg/ml. Respective solvents served as control. They were incubated at 37°C for 24 hours. After incubation, the diameter of the inhibition zone was measured.

Result and Discussion

Phytochemical screening

The methanolic extracts of five medicinal plants were subjected to preliminary phytochemical screening of various constituents such as flavanoids, alkaloids, glycosides, proteins, carbohydrates, tannins, saponins, triterpenoids, anthraquinones and phytosterols in the leaves.

Antibacterial activity of Plant extracts

Four solvents such as methanol,

chloroform, ethyl acetate and aqueous were used to the extraction of leaf samples of plants. Among these various extracts used for screening of antibacterial activity of these five plants, in which the methanolic extracts shows (Table-2) the most effective in the inhibiting the growth of *E.coli*, *K.pneumoniae*, *M.luteus* and *P.aeruginosa*. Aqueous, ethyl acetate, and chloroform extracts showed lesser activity one another respectively. *Cassia alata* and *Cardiospermum halicacabum*) showed maximum activity than other plants. *E. coli* and *M. luteus* more susceptible for all extracts than others.

Table.1 Phytochemical screening of five Indian medicinal plants (Methanolic extract)

Phytochemicals Plants	<i>A.Paniculata</i>	<i>C.alata</i>	<i>C.halicacabum</i>	<i>N.arbortritis</i>	<i>S.nigrum</i>
Alkaloids	+	+	+	+	+
Flavonoids	+	+	+	+	-
Glycosides	+	+	+	+	+
Saponins	-	-	+	+	+
Phytosterols	+	+	+	+	+
Triterpenoids	+	+	+	-	+
Tannins	-	+	-	-	-
Proteins	+	+	+	+	+
Carbohydrates	+	+	+	+	+
Anthraquinones	-	+	-	-	-

+ = Presence of phytochemicals; - = Absence of phytochemicals

Table.2 Effect of methanolic extract (100 µl) of five Indian medicinal plants against human Pathogens (Diameter of inhibition zone in mm)

Plants / Bacteria	<i>E.coli</i>	<i>K.pneumoniae</i>	<i>M.luteus</i>	<i>P.aeruginosa</i>
<i>A.paniculata</i>	16	15	18	15
<i>C.alata</i>	19	12	20	14
<i>C.halicacabum</i>	20	13	19	15
<i>N.arbortritis</i>	18	14	18	16
<i>S.nigrum</i>	14	10	16	14

Plate.1 Antibacterial activity of *A. paniculata*



Plate. 2 Antibacterial activity of *C. alata*



Available literature indicated that medicinal plants are the backbone of traditional medicine and the antibacterial activity of plant extract is due to different chemical agent in the extract which was classified as active antimicrobial compounds (Arulmozhi *et al.*, 2007). Alkaloids are formed as metabolic byproducts and have been reported to be responsible for the antibacterial activity (Mantle *et al.*, 2000). Tannins have been found to form irreversible complexes with proline rich proteins, resulting in the inhibition of the cell protein synthesis. This activity was exhibited against test organisms with all plant extracts. Tannins have important roles such as stable and potent antioxidant. Flavonoids are phenolic structures containing one carbonyl group. Flavonoid complexes with extra cellular and soluble protein and with bacterial cell wall. Thus, they exhibit antibacterial activity (Balasubramanian, 2012). Glycosides serve as defence mechanisms against predation by many microorganisms, insects and herbivores (Dhar *et al.*, 1979.). The demonstration of antimicrobial activity against both gram positive and gram negative bacteria by the plant may be indicative of the presence of broad spectrum of antibiotic compounds

(Lans, *et al.*, 2001). The optimal effectiveness of a medicinal plant may not be due to the one main active constituent, but may be due to the combined action of different compounds originally in the plant (Bhandarkar *et al.*, 2003).

Preliminary phytochemical analysis revealed the presence of alkaloids, saponins, other secondary metabolites like tannins, flavonoids, steroids, cardiac glycosides, etc. were present in these five plants. Some of the chemical constituents absent in some plants, It is not surprising that there are differences in the antimicrobial effects of plant species, due to the phytochemical properties and differences among species. . It is quite possible that some of the plants that were less effective in this study may not possess antibiotic properties, or the plant extracts may have contained antibacterial constituents, just not in sufficient concentrations, so as to be effective. It is also possible that the active chemical constituents were not soluble in methanol or water. The drying process may have caused conformational changes to occur in some of the chemical constituents found in these plants.

In our present findings, four solvents such as methanol, chloroform, ethyl acetate and aqueous were used to the extraction of leaf samples of *Andrographis paniculata*, *Cassia alata*, *Cardiospermum halicacabum*, *Nyctanthes arbortritis* and *Solanum nigrum*.

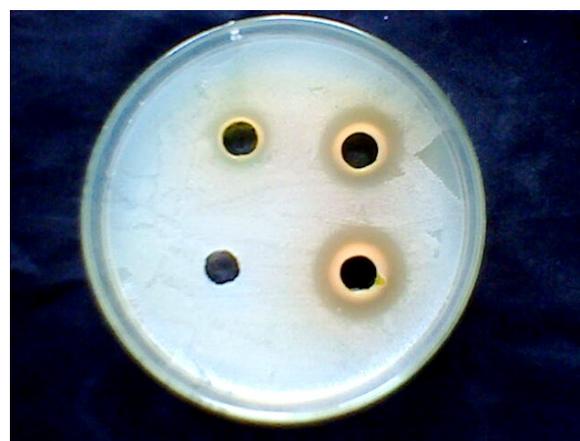
Among these various extracts used for screening of antibacterial activity of these five plants, in which the methanolic extracts shows the most effective in the inhibiting the growth of *Escherichia coli*, *Klebsiella pneumoniae*, *Micrococcus luteus* and *Pseudomonas aeruginosa*.

Methanolic extract of *C. alata* showed maximum inhibition zone (20mm) against *M. luteus*, *C. halicacabum*, showed maximum zone (20 mm) against *E. coli* *N. arbortritis*, and *A. paniculata* were showed maximum zone against, *P. aeruginosa* (16 mm), and *K. pneumoniae* (15 mm) respectively.

Plate.3 Antibacterial activity of *B. halicacabum*



Plate.4 Antibacterial activity of *S. nigrum*



In the present study screening of different plant species, the results obtained confirm the therapeutic potency of some plants used in traditional medicine. In addition, these results form a good basis for

selection of candidate plant species for further phytochemical and pharmacological investigation. The results of the present study support the folkloric usage of the studied plants and suggest that some of the plant extracts possess compounds with antibacterial properties that can be used as antimicrobial agents in new drugs for the therapy of infectious diseases caused by pathogens. The most active extracts can be subjected to isolation of the therapeutic antimicrobials and undergo further pharmacological evaluation.

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